

**A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, 9-MONTH, PARALLELL GROUP STUDY OF ALLOPURINOL TO REDUCE LEFT VENTRICULAR MASS IN LIVING KIDNEY DONORS (AL-DON)**

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*I hereby declare that I will conduct the study in compliance with the Protocol, ICH GCP and the applicable regulatory requirements:*

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# PROTOCOL SYNOPSIS

A randomized, double-blind, placebo-controlled, 9-month, parallel group study of allopurinol to reduce left ventricular mass in living kidney donors (AL-DON)

Sponsor	Oslo University Hospital, Rikshospitalet, Oslo, Norway.
Phase and study type	Phase IIb interventional efficacy study
Investigational Medical Product (IMP) (including active comparator and placebo) :	Allopurinol and placebo
Centers:	Oslo University Hospital, Rikshospitalet
Study Period:	Estimated date of first patient enrolled: 01.08.2017 Anticipated recruitment period: 3 years Estimated date of last patient completed: 31.12.2021
Treatment Duration:	9 months
Follow-up:	9 months
Objectives	Evaluate if allopurinol lowers left ventricular mass in living kidney donors  Key secondary objectives are to evaluate if allopurinol lowers blood pressure and increases insulin sensitivity
Endpoints:	Primary endpoint: Change in left ventricular mass from baseline to month 9 in the allopurinol group compared to placebo  Secondary endpoints: Change from baseline to month 9 in the allopurinol group compared to placebo in systolic and diastolic ambulatory blood pressure, systolic and diastolic office blood pressure, estimated metabolic clearance rate of glucose, estimated insulin sensitivity, estimated first-phase insulin release, estimated second-phase insulin release, serum uric acid levels, number and doses of antihypertensive medications, urinary albumin excretion rate, estimated glomerular filtration rate.

Study Design:	The study is a single-centre, phase 2b efficacy, 1:1 randomized, double-blind, placebo-controlled, 9-month, 2 arm parallel group, interventional trial to assess the superiority of allopurinol vs. placebo on reduction of left ventricular mass in living kidney donors.
Main Inclusion Criteria:	<ol style="list-style-type: none"> <li>1. Kidney donor <math>\geq 6</math> months after donor nephrectomy</li> <li>2. Donor nephrectomy undertaken in Norway</li> <li>3. Male or female subject <math>\geq 18</math> years old</li> <li>4. eGFR <math>&gt;30</math> ml/min/1.73 m<sup>2</sup></li> <li>5. Signed informed consent and expected cooperation of the patients for the treatment and follow up must be obtained and documented according to ICH GCP, and national/local regulations.</li> </ol>
Main Exclusion Criteria	<ol style="list-style-type: none"> <li>1. Adverse reactions to allopurinol or other xanthine oxidase inhibitors</li> <li>2. Use of uric acid lowering therapy within 3 months</li> <li>3. History of gout, xanthinuria or other indications for uric acid lowering therapy such as cancer chemotherapy</li> <li>4. History of renal calculi</li> <li>5. History of coronary heart disease</li> <li>6. Heart failure with left ventricular ejection fraction <math>&lt;45\%</math></li> <li>7. History of significant (i.e. non-physiological) cardiac valvular stenosis or insufficiency</li> <li>8. History of clinically significant hepatic disease including hepatitis B or C and/or ALAT (SGPT) above the upper reference limit at screening.</li> <li>9. History of HIV or AIDS</li> <li>10. Severe systemic infections, current or within the last 6 months</li> <li>11. History of malignancy other than localized basal cell carcinoma of the skin, treated or untreated, within the past 5 years.</li> <li>12. Other life-threatening diseases</li> <li>13. Haemoglobin concentration <math>&lt; 11</math> g/dL(males), <math>&lt;10</math> g/dL (females); white blood cell (WBC) count <math>&lt; 3.5 \times 10^9/L</math>; platelet count <math>&lt;50 \times 10^9/L</math> at screening</li> <li>14. Use of the following medications at or within 14 days before the screening visit: azathioprine, mercaptopurine, vidarabin, chlorpropamide, warfarin, tamoxifen, theophylline, amoxicillin/ampicillin, cyclophosphamide, doksorubicin, bleomycin, prokarbazin, cyclosporine, didanosine.</li> <li>15. Contraindications to MRI, including: Magnetic intracranial clips. Metal fragments in orbita. Cochlea (ear) implant. Neurostimulator. Pacemaker/ICD or remaining pacemaker electrodes. Harrington rods in thorax. Claustrophobia. Unable to lie supine.</li> </ol>

16. Pregnant or nursing (lactating) women
17. Fertile women, unless they are using effective contraception during dosing of study treatment
18. Any reason why, in the opinion of the investigator, the patient should not participate.

Sample Size: 80 patients

Efficacy Assessments: Left ventricular mass determined by MR cor. 24-h ambulatory blood pressure and office blood pressure. Insulin sensitivity and insulin release estimated by oral glucose tolerance test according to Stumvoll et al (1). Blood sample analysis of uric acid, glucose, insulin, estimated glomerular filtration rate (creatinine-based CKD-EPI formula (2)), cholesterol fractions and triglycerides. Urine albumin-creatinine ratio. Record of number of antihypertensive medications.

Safety Assessments: All participants will be told to stop study drug and call the study staff in case of fever or rash including lesions in mucous membranes. Planned assessment of blood sample at day  $14 \pm 7$  including full blood count, renal and liver function tests.

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## LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or special term	Explanation
AE	Adverse Event
AL-DON	Short name of this study
BP	Blood Pressure
CRF	Case Report Form (paper)
CSA	Clinical Study Agreement
CSR	Clinical Study Report
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Event
DAE	Discontinuation due to Adverse Event
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
GCP	Good Clinical Practice
HOMA-IR	Homeostatic Model Assessment-Insulin Resistance
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product (includes active comparator and placebo)
IND	Investigational New Drug
ITT	Intention To Treat
LVM	Left Ventricular Mass
MRI	Magnetic Resonance Imaging
OGTT	Oral Glucose Tolerance Test
PI	Principal Investigator
PP	Per Protocol
SAE	Serious Adverse Event
SD	Stable Disease
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
XOR	Xanthine dehydrogenase/Oxidoreductase

# 1 INTRODUCTION

## 1.1 Background – Cardiovascular risk in living kidney donors

*Key studies regarding left ventricular hypertrophy in living kidney donors and randomized trials of allopurinol on left ventricular mass, blood pressure and insulin resistance are summarized in Appendix A.*

### *Long-term survival in kidney donors*

Kidney donation has long been considered relatively safe, with no impact on the longevity of the donor. In support of this view, previous observational studies found similar or better survival in kidney donors compared to the general population (3), and similar survival compared to healthy controls over short-term follow-up (< 8 years; (4-6).

In contrast, we recently reported a long-term follow-up study of Norwegian kidney donors, in which we compared their survival to healthy controls from the Health Survey in North Trøndelag (HUNT) (7). After a median follow-up of 15.1 years, the kidney donors experienced a significant increase in both cardiovascular mortality (hazard ratio 1.40 [95% confidence interval 1.03-1.91]), and all-cause mortality (HR 1.30 [1.11-1.52]) and a pronounced increased risk of end-stage renal disease (HR 11.4 [4.4-29.6]). Some of this increased risk may be explained by shared genetics, since 80% of donors were first-degree relatives of the recipient, and such genetic relationship has been associated with risk of end-stage kidney disease and mortality even in non-donors (8). Even though our study does not prove causality from donation, this mortality risk resembles what has been found in observational studies in the general population with similar reductions in kidney function (9), which may suggest causal effects. Thus, our findings raise concern for the long-term health risks in kidney donors and highlight the need for strategies to mitigate this risk.

### *Cardiovascular risk factors in kidney donors*

Several modifiable cardiovascular risk factors in kidney donors have recently been identified. Moody et al. compared risk factors in 124 kidney donors and healthy controls over 12 months after donation, and found that donors had significant increase in left ventricular mass and several biochemical parameters including serum uric acid, parathyroid hormone, fibroblast growth factor-23, C-reactive protein and increased odds of detectable troponin T and micro-albuminuria (10). They also found that the reduction in GFR was associated with the increase in left ventricular mass, suggesting that the reduced GFR was a causative cardiovascular risk factor. Altmann et al also recently found increased LVM 1 year after kidney donation, albeit to a lesser degree than Moody et al (11). Previous studies also found an increase in blood pressure in donors (12, 13), although this was not seen in the latest studies by Moody et al and Altmann et al (10, 11). Few studies describe insulin resistance in kidney donors. A cross-sectional study found increased homeostasis model assessment of insulin resistance in 14 donors vs. 25 healthy controls (14). An American study found that metabolic syndrome in donors was associated with a protracted recovery of kidney function (15).

### *Left ventricular hypertrophy (LVH)*

LVH is a major cardiovascular risk factor. In the Framingham Heart study, LVH was second only to age as a predictor of cardiovascular events, cardiovascular death and total mortality (16). Similarly, LVH is a strong risk factor in patients with chronic kidney disease (17). Control of blood pressure by antihypertensive agents has been shown to regress LVH, other treatment options include weight loss and sodium restriction (18, 19). LVH is increasingly prevalent in higher stages of chronic kidney disease (CKD), and present in most patients on dialysis, indicating that renal dysfunction may be a causal factor (20). The finding by Moody et al. of an increased left ventricular mass without an elevation in blood pressure supports this causality (10). Notably, regression of LVH is associated with improved prognosis, independently of changes in blood pressure (21, 22). This indicates that therapies directed at LVH beyond control of blood pressure could be of benefit.

### *Uric acid*

Uric acid lowering therapy may be a new strategy to improve LVH. Recent studies in patients with CKD (23), type 2 diabetes (24) and ischemic heart disease (25) found regression in left ventricular mass with allopurinol. As uric acid levels increase after kidney donation (10), such a strategy may be of particular value in kidney donors.

Uric acid has been debated for more than 130 years as a causal factor in human cardiovascular and renal disease (26). Studies in rodents undertaken since year 2000 found that uric acid which is increased experimentally, causes hypertension, metabolic syndrome and kidney disease (27-29). Indeed, uric acid fulfils both the causal Bradford-Hill criteria and the Henle-Kochs postulates for rodent hypertension (30). A landmark study by Feig et al. in 2008

documented that blocking uric acid synthesis with allopurinol lowers blood pressure in human hypertensive adolescents as well (31). In a later study, probenecid, a uricosuric drug, lowered blood pressure to a similar extent as allopurinol (32).

Uric acid is the final break-down product of purines in man (from DNA, RNA, ATP, cAMP), two thirds is excreted through the kidney in a complex process involving glomerular filtration and tubular reabsorption and secretion, the rest through the gut. The last two steps in the synthesis is catalyzed by the enzyme xanthine dehydrogenase/oxidoreductase (XOR), which under certain conditions (ie ischemia) may donate electrons to molecular oxygen, yielding superoxide, a free radical (33). XOR is distributed in various organs including the liver, gut, lung, kidney, heart, brain and plasma and the capillary endothelial cells of the myocardium. Most mammals except human and great apes possess uricase which further breaks down uric acid to allantoin, a more water soluble molecule. Uric acid is an extracellular antioxidant, and evolutionary benefits of the higher human uric acid levels have been discussed (34). Uric acid levels can be increased by increased alimentary intake, increased production or reduced elimination. The production increases in states of catabolism or tissue break down, as in cancer chemotherapy, and by hypoxia, limb ischemia, major surgery, coronary artery disease and heart failure (35). The renal excretion of uric acid is reduced in kidney failure or by drugs such as diuretics and calcineurin-inhibitors (36). Uric acid and/or XOR activity impairs endothelial cell function, stimulates vascular smooth muscle cell proliferation, stimulates inflammation and upregulates the renin-angiotensin system (26, 35). In rodents uric acid stimulates inflammation in adipose tissue and induces insulin resistance, which is reversible by allopurinol (37).

## 1.2 Background - Therapeutic Information

Kidney donors are followed clinically on a regular basis. Norwegian donors are seen by their local nephrologist at 3 months, 6 months and 1 year after nephrectomy, then yearly up to 5 years and thereafter every fifth year. There is a particular emphasis on blood pressure, renal function and proteinuria, and cardiovascular drugs are prescribed for similar indications as in the general population. There are no current recommendations for uric acid lowering therapy other than for patients with gout or certain types of kidney stones, as in the general population.

Potential cardiovascular benefits of allopurinol have gained attention during the last two decades and been extensively reviewed (38, 39). Key human pilot studies regarding hypertension, left ventricular mass and insulin resistance are summarized in Appendix A.

Allopurinol was initially developed as an anti-neoplastic agent, but the ability to reduce uric acid by inhibition of xanthine dehydrogenase/oxidoreductase (XOR) led to its use in gout and hyperuricemia. Allopurinol is a competitive inhibitor of XOR at low doses and noncompetitive at high levels. It is metabolized by XOR to oxypurinol which also is a noncompetitive inhibitor of XOR; direct free radical scavenging effects have also been described (38). Allopurinol improves endothelial dysfunction in a dose-dependent manner by reducing oxidative stress, whereas probenecid does not (40). The highest dose (600 mg allopurinol) completely abolished residual oxidative stress (as evaluated by vitamin C co-infusion), and was suggested for future studies of XOR inhibition in reducing cardiovascular events (39, 40).

## 1.3 Pre-Clinical & Clinical Experience with Investigational Medicinal Product (IMP)

The studies below are summarized in Appendix A.

Proof-of-principle in human of a blood pressure (BP) lowering effect of allopurinol was provided by Feig et al in 2008 (31). In this trial, 30 adolescents with newly diagnosed, never-treated hypertension and uric acid levels  $\geq 357 \mu\text{mol/L}$  were included in a randomized cross-over trial of allopurinol 200 mg x 2 vs placebo. Treatment and placebo were administered for 4 weeks each, with an intervening 2 weeks wash-out period. For casual BP, the mean change in systolic BP for allopurinol was -6.9 mmHg vs -2.0 mmHg for placebo ( $p<0.009$ ), and the mean change in diastolic BP for allopurinol was -5.1 mmHg vs -2.4 for placebo ( $p=0.05$ ). Similarly, for 24-hour ambulatory BP, the mean change in systolic BP for allopurinol was -6.3 mmHg vs 0.8 mmHg for placebo ( $p=0.001$ ) and mean change in diastolic BP for allopurinol -4.6 mmHg vs -0.3 mmHg for placebo ( $p=0.004$ ). These reductions in BP were similar to what is achieved with standard antihypertensive medications. Systemic vascular resistance and plasma renin activity decreased with treatment, pointing to a mechanism by which allopurinol lowers BP. Animal studies support this mechanism, by demonstrating that uric acid stimulates renin expression (27).

A question raised by the latter study was if the effect of allopurinol on BP was caused by a reduction in uric acid *per se* or by an inhibition of xanthine oxidase and superoxide production. In 2012, the same authors therefore tested whether

probenecid, a uricosuric drug, could be similarly effective as allopurinol to reduce BP (32). This latter study included 60 obese pre-hypertensive adolescents, randomized to allopurinol, probenecid or placebo for 8 weeks (n=20 in each group; double-blinded). The first week allopurinol was administered 100 mg x 2 (i.e. twice daily) and probenecid 250 mg x 2, the remaining 7 weeks allopurinol was administered 200 mg x 2 and probenecid 500 mg x 2. At the end of treatment, BP was similarly lowered in both treatment groups, with a fall in systolic BP of about 10 mmHg and diastolic BP of about 8 mmHg, significantly different from the placebo group in which BP was unchanged. This study indicates that lowering uric acid *per se* could rectify pre-hypertension.

Effects of allopurinol on several cardiovascular risk factors in humans were also described in a recent Chinese study, published in 2015 by Liu *et al* (41). The primary endpoint was the carotid intima-media thickness, secondary endpoints included blood pressure, insulin resistance (assessed by homeostatic model assessment-insulin resistance [HOMA-IR]) and hs-CRP. Renal function and blood lipids were also reported. This was a 3-year open-labeled randomized study of allopurinol, and included 176 patients with type 2 diabetes and asymptomatic hyperuricemia (serum uric acid between 420 to 476  $\mu\text{mol/L}$ ), excluding patients with gout, hypertension, renal insufficiency, severe cardiac, hepatic or cerebral disease, or use of certain medications, including ACE-inhibitors, angiotensin receptor blockers or drugs affecting uric acid levels. In the intervention arm allopurinol was given if uric acid levels exceeded 420  $\mu\text{mol/L}$ , starting at 100 mg and titrated to achieve uric acid levels below 360  $\mu\text{mol/L}$  (dose range 100 to 450 mg/day, average  $234 \pm 87$  mg/day). Uric acid was lowered by  $104 \pm 19$   $\mu\text{mol/L}$  in the allopurinol group (to  $329 \pm 19$   $\mu\text{mol/L}$ ), but was unaltered in the control group at  $455 \pm 12$   $\mu\text{mol/L}$ . Compared to controls, the allopurinol group had less progression in carotid intima-media thickness ( $0.02 \pm 0.03$  vs  $0.07 \pm 0.03$  mm;  $p < 0.001$ ), less increase in blood pressure (rise in systolic BP  $2 \pm 3$  vs  $6 \pm 2$  mmHg,  $p < 0.001$ ; rise in diastolic BP  $1 \pm 3$  vs  $4 \pm 2$  mmHg,  $p < 0.001$ ), and non-significantly less incident hypertension (6.8% vs 13.6%,  $p = 0.14$ ). Insulin resistance as assessed by HOMA-IR increased less ( $0.10 \pm 0.15$  vs  $0.21 \pm 0.19$ ;  $p < 0.001$ ), but fasting glucose, 2h postprandial glucose and HbA1c were unchanged (all  $p > 0.59$ ). hs-CRP increased less ( $0.15 \pm 0.19$  vs  $0.35 \pm 0.19$ ;  $p < 0.001$ ). HDL was less reduced ( $-0.02 \pm 0.03$  vs  $-0.04 \pm 0.03$ ;  $p = 0.001$ ), triglycerides increased less ( $0.12 \pm 0.12$  vs  $0.29 \pm 0.16$ ;  $p < 0.001$ ), whereas LDL and total-cholesterol levels were unaltered ( $p > 0.82$ ). eGFR was also more preserved ( $-0.8 \pm 3.9$  vs  $-4.9 \pm 5.0$  mL/min/1.73m<sup>2</sup>;  $p < 0.001$ ) in the allopurinol group.

Three studies have described reduction in MR cor- derived left ventricular mass with allopurinol treatment (23-25). The first of these studies, published in 2011 by Kao *et al*, randomized 67 patients with stage 3 CKD and LVH to 300 mg allopurinol or placebo for 9 months, of whom 53 patients completed the study (allopurinol, n=27; placebo, n=26). Left ventricular mass indexed to body surface area (LVMI) was reduced in the allopurinol group ( $-1.42 \pm 4.67$  g/m<sup>2</sup>) compared with the placebo group ( $+1.28 \pm 4.45$  g/m<sup>2</sup>),  $p = 0.036$ , i.e. a difference in change of  $-2.7$  g/m<sup>2</sup>. LVM change in absolute values (i.e. not indexed for BSA) was not reported. A later study in patients with ischemic heart disease and LVH randomized 66 patients to 600 mg (300 mg x 2) allopurinol or placebo for 9 months, of whom 55 completed the study (allopurinol, n=27; placebo, n=28). Left ventricular mass was reduced in the allopurinol group ( $-5.2 \pm 5.8$  g) compared with the placebo group ( $-1.3 \pm 4.48$  g),  $p = 0.007$ , i.e. a difference in change of  $-3.89$  g (95% CI  $-1.1$  to  $-6.7$ ). A similar study in patients with type 2 diabetes and LVH randomized 66 patients to 600 mg (300 mg x 2) allopurinol or placebo for 9 months, of whom 55 completed the study (allopurinol, n=26; placebo, n=29). Left ventricular mass was reduced in the allopurinol group ( $-2.65 \pm 5.91$  g) compared with the placebo group ( $+1.21 \pm 5.10$  g),  $p = 0.012$ , i.e. a difference in change of  $-3.89$  g (95% CI  $-1.1$  to  $-6.7$ ), i.e. a difference in change of  $-3.86$  g. None of these studies found significant changes in blood pressure. In summary, 9 months of allopurinol was associated with a similar regression in LVM of about 3.9 g.

In addition to the afore mentioned effects on blood pressure and left ventricular mass, pilot studies with allopurinol suggest renoprotection (42-46), anti-ischemic properties in angina pectoris (47) and improved myocardial efficiency in dilated cardiomyopathy (48). Some discrepant studies exist regarding cardiovascular benefit of allopurinol. Two studies in patients with acute myocardial infarction came to different conclusions regarding the resulting infarct size (49, 50). A study in 405 heart failure patients failed to show clinical improvements, although post-hoc analysis suggested benefits in patients with elevated uric acid in a manner correlating with the degree of uric acid reduction (51). A later study of allopurinol in 253 heart failure patients with hyperuricemia ( $>565$   $\mu\text{mol/L}$ ) failed to demonstrate improvement in clinical status, exercise capacity, quality of life or left ventricular ejection fraction within 24 weeks of treatment (52).

## 1.4 Rationale for the Study and Purpose

This project aims to clarify potential benefit to kidney donors of allopurinol treatment regarding three surrogate cardiovascular endpoints; left ventricular mass, blood pressure and insulin sensitivity. Improvements in these endpoints

would be an indication for a lower risk for cardiovascular disease and diabetes, but would need to be verified in larger studies.

Regarding the design, the main advantage of a randomized experiment (vs. an observational study) is to balance both measured and unmeasured confounding variables. Blinded placebo control is used to create a more valid comparator group. This is particularly important since a differential behaviour between the study arms, for instance a differential change in diet, may create a bias. Blinding of the study staff is also important for unbiased measurements.

Regarding the dose of allopurinol, as described in Section 1.2, 600 mg was suggested in a study on oxidative stress (39, 40), and two of the three former trials of allopurinol and left ventricular mass used this dose (23-25). In patients with reduced kidney function the active metabolite oxypurinol may accumulate, and the study on left ventricular mass in patients with CKD used a dose of 300 mg (23). We therefore plan to use 300 mg of allopurinol.

## **2 STUDY OBJECTIVES AND RELATED ENDPOINTS**

### **2.1 Primary Endpoint**

Change in left ventricular mass measured by cardiac MRI from baseline to month 9 in the allopurinol group compared to placebo

### **2.2 Secondary Endpoints**

Change from baseline to month 9 in the allopurinol group compared to placebo for the following parameters

- Systolic and diastolic ambulatory blood pressure, and systolic and diastolic office blood pressure
- Insulin sensitivity estimated according to Stumvoll et al using the oral glucose tolerance test (1), i.e. estimated metabolic clearance rate of glucose and estimated insulin sensitivity index
- 1st and 2nd phase insulin release estimated according to Stumvoll et al using the oral glucose tolerance test (1)
- Other secondary variables: Blood sample analysis of uric acid, glucose, insulin, estimated glomerular filtration rate (creatinine-based CKD-EPI formula (2)), cholesterol fractions and triglycerides. Urine albumin-creatinine ratio. Number of antihypertensive medications.

### 3 OVERALL STUDY DESIGN

The study is a single-centre, phase 2b efficacy, 1:1 randomized, double-blind, placebo-controlled, 9-month, 2 arm parallel group, interventional trial to assess the superiority of allopurinol vs. placebo on reduction of left ventricular mass in living kidney donors.

Study Period Estimated date of first patient enrolled: 01.08.2017

Anticipated recruitment period: 3 years

Estimated date of last patient completed: 31.12.2021

Treatment Duration: 9 months

Follow-up: 9 months

### 4 STUDY POPULATION

#### 4.1 Selection of Study Population

Living kidney donors. Recruitment will start among donors residing near Oslo University Hospital, Rikshospitalet.

#### 4.2 Number of Patients

The target is to include 80 living kidney donors.

#### 4.3 Inclusion Criteria

All of the following conditions must apply to the prospective patient at screening prior to receiving study agent (e.g.):

1. Kidney donor  $\geq$  6 months after donor nephrectomy
2. Donor nephrectomy undertaken in Norway
3. Male or female subject  $\geq$  18 years old
4. Signed informed consent and expected cooperation of the patients for the treatment and follow up must be obtained and documented according to ICH GCP, and national/local regulations.

#### 4.4 Exclusion Criteria

Patients will be excluded from the study if they meet any of the following criteria:

1. Adverse reactions to allopurinol or other xanthine oxidase inhibitors
2. Use of uric acid lowering therapy within 3 months
3. History of gout, xanthinuria or other indications for uric acid lowering therapy such as cancer chemotherapy
4. History of renal calculi

5. History of coronary heart disease
6. Heart failure with left ventricular ejection fraction <45%
7. History of significant (i.e. non-physiological) cardiac valvular stenosis or insufficiency
8. History of clinically significant hepatic disease including hepatitis B or C and/or ALAT (SGPT) above the upper reference limit at screening.
9. History of HIV or AIDS
10. Severe systemic infections, current or within the last 6 months
11. History of malignancy other than localized basal cell carcinoma of the skin, treated or untreated, within the past 5 years.
12. Other life-threatening diseases
13. Haemoglobin concentration < 11 g/dL (males), <10 g/dL (females); white blood cell (WBC) count <  $3.5 \times 10^9/L$ ; platelet count <  $50 \times 10^9/L$  at screening
14. Use of the following medications at or within 14 days before the screening visit: azathioprine, mercaptopurine, vidarabin, chlorpropamide, warfarin, tamoxifen, theophylline, amoxicillin/ampicillin, cyclophosphamide, doksorubicin, bleomycin, prokarbazin, cyclosporine, didanosine.
15. Contraindications to MRI, including: Magnetic intracranial clip. Metal fragment in orbita. Cochlea (ear) implant. Neurostimulator. Pacemaker/ICD or remaining pacemaker electrode. Harrington rod in thorax. Claustrophobia. Unable to lie supine.
16. Pregnant or nursing (lactating) women
17. Fertile women, unless they are using effective contraception during dosing of study treatment
18. Any reason why, in the opinion of the investigator, the patient should not participate.

## 5 TREATMENT

For this study allopurinol and placebo are defined as Investigational Medicinal Product(s) (IMP).

### 5.1 Drug Identity, Supply and Storage

Allopurinol 300 mg and corresponding placebo.

Allopurinol tablets will be purchased commercially from Takeda (Allopur 100 mg), while placebo will be made at Kragerø Tablettproduksjon AS. Both active study drug and placebo will be over encapsulated to assure identical appearance.

Storage and preparation of both allopurinol and placebo (IMP) will be according to the package leaflet for allopurinol.

### 5.2 Dosage and Drug Administration

Capsules containing allopurinol 300 mg or matching placebo (IMP) are taken by mouth once daily. The capsule is preferably taken with food and swallowed whole with water. If a dose is missed, it should be taken as soon as the trial



participant remembers until 12 hours after scheduled intake, after that the patient should wait until next scheduled dose.. A double dose should not be taken on the same day.

### **5.3 Duration of Therapy**

The intended duration of treatment with allopurinol or matching placebo is 9 months (acceptable range 8-11 months). Treatment should continue up to and including the day of assessment of outcome data, unless unacceptable toxicity is encountered.

Study subjects may also discontinue protocol therapy in the following instances:

- Intercurrent illness which would in the judgment of the investigator effect patient safety, the ability to deliver treatment or the primary study endpoints
- Request by patient

### **5.4 Schedule Modifications**

In case of unacceptable toxicity the IMP is discontinued

In case of minor side effects potentially related to treatment, the IMP may also be discontinued. If the symptoms resolve within 21 days, the IMP may be re-introduced. If IMP is discontinued >21 days the study subject will be defined as poor adherent (Section 5.6), and need not resume the IMP treatment.

### **5.5 Concomitant Medication**

The following medication is not allowed while the patient is in the treatment phase of the study:

Medications mentioned as exclusion criteria (Section 4.4 pt 29): azathioprine, mercaptopurine, other immunosuppressive therapy, warfarin, ampicillin, theophylline, chlorpropamide, vidarabine, didanosien, cytotoxic drugs.

All concomitant medication (incl. "over-the-counter" drugs) used by the patient will be recorded in the patient's file and CRF.

### **5.6 Subject Compliance**

The procedure for determining adherence in this study is to ask the patients to write a medical diary of the doses taken. Poor adherence is defined if the IMP is taken in less than 80% of the treatment period or if IMP is discontinued for more than 21 subsequent days. Patients with poor adherence will be regarded as part of the ITT population, but not the PP population. An estimate of remaining capsules will also be performed at study end from returned used and unused pill boxes.

### **5.7 Drug Accountability**

The responsible site personnel will confirm receipt of study drug and will use the study drug only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return, and destruction (if any) of the study drug must be properly documented according to the sponsor's agreed and specified procedures.

Active study drug will be purchased commercially, while placebo will be made at Kragerø Tablettproduksjon AS. Both active study drug and placebo will be over encapsulated in order to maintain the blinding. Kragerø Tablettproduksjon AS will perform the production of study medication and provide the details on drug accountability. The IMP will be delivered from Kragerø Tablettfabrikk to the study investigator at Oslo University Hospital, Rikshospitalet and documented in the investigator site file. The IMP will be stored in a locked cabinet at a maximum of 25 degrees Celcius. Administration of drug will be in accordance with numbered envelopes containing the number of the allocated drug package, which will be recorded in the CRF. Trial participants will be asked to write a medical diary of the doses taken. At the end of study, used and unused pill boxes will be returned to the study staff, and remaining capsules will be estimated. Unused

medicinal product or waste material will be disposed of in accordance with local requirements, i.e. delivered to pharmacy for destruction.

## **5.8 Drug Labeling**

The investigational product will have a label permanently affixed to the outside and will be labeled according with ICH GCP and national regulations, stating that the material is for clinical trial / investigational use only and should be kept out of reach of children. Label layout will be provided by Kragerø Tablettproduksjon AS.

Labels will also include the following information (in Norwegian) for:

- Protocol code
- Name of Principal Investigator
- Patient's enrolment code
- Patient's initials
- Delivery date
- Expiration date
- Batch number

## **5.9 Subject Numbering**

Each subject is identified in the study by a unique subject number that is assigned when subject signs the Informed Consent Form. Once assigned the subject number cannot be reused for any other subject.

The study treatment will be dispensed to the subject by authorized site personnel only.

# **6 STUDY PROCEDURES**

## **6.1 Flow Chart (Table 1; next page)**

**Table 1. Trial flow chart**

	Screening/baseline		Treatment Period			End of treatment visit <sup>7</sup>		Withdrawal visit (if applicable)	
Time	Screening	Baseline <sup>5</sup>	D1	D 14±7		D 275 (-30 to +60)		Dx	
Informed consent	X								
Inclusion/exclusion Evaluation	X								
Medical History	X	X							
Physical Examination	X	X				X		X	
Vital signs <sup>1)</sup>	X	X				X		X	
Blood samples <sup>2)</sup>	X	X		X		X		X	
Urine samples <sup>3)</sup>		X				X		X	
Biobanking of blood		X				X			
Pregnancy test	X								
Randomization		X							
Treatment dispensation		X							
Cardiac MRI		X				X			
OGTT		X				X			
24h blood pressure		X	(X) <sup>6</sup>			X			
Adverse event <sup>4</sup>				X		X		X	
Record of concomitant medication & drug diary	X	X		X		X		X	

1. Blood pressure, pulse, temperature, weight.

2. CRP, Hb, WBC (incl.differential counting), PLT, Na, K, Ca, creatinine, ASAT, ALAT, LD, ALP, γGT, INR, albumin, bilirubin.

3. Urine for stix (glucose, blood and protein) and albumin-creatinine ratio.

4. Notably skin eruptions, infections, abdominal pain, nausea, leucopenia, pathologic liver function tests

5. Screening results can be used as baseline values if screening was undertaken within 21 days prior to treatment. All procedures need not be on the same day.

6. If baseline 24h BP record ends on D1, the investigational medicinal product (IMP; i.e. the study drug) should be started *after* the 24h BP record has finished.

7. All procedures need not be on the same day, but should preferably be undertaken while trial participant is still taking the IMP.

## 6.2 By Visit

### 6.2.1 Before Treatment Starts

#### Visit 1: Consent and screening.

##### **Informed consent**

- Informed consent must have been given voluntarily by each subject before any study specific procedures are initiated. The following tests will be done at screening, and may be used as baseline values as long as  $\leq 21$  days prior to treatment start.

##### **Clinical status**

- Medical history (including disease history and corresponding treatment details), physical examination eg. (cor/pulm/abdomen and peripheral lymph node status), vital signs (weight, blood pressure, temperature and pulse)

##### **Concomitant medication**

- All concomitant medication (incl. "over-the-counter" drugs) used by the subject within 14 days before screening visit must be recorded in the CRF.

##### **Laboratory analysis**

- Blood samples will be taken to determine absence of exclusion criteria regarding renal function, hematology or liver function: CRP, Hb, WBC (incl. differential counting), PLT, Na, K, Ca, creatinine, ASAT, ALAT, LD, ALP,  $\gamma$ GT, INR, albumin, bilirubin. In addition: cholesterol fractions and triglycerides.

##### **Women of childbearing potential**

A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

Inclusion of WOCBP will only be performed after a negative highly sensitive pregnancy test. The inclusion of WOCBP requires use of a highly effective contraceptive measure. Such methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - oral
  - intravaginal
  - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable
  - implantable
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised partner
- sexual abstinence

Contraception should be maintained during treatment and until the end of relevant systemic exposure (90 days after study completion). All eligibility criteria should be assessed together with relevant baseline parameters prior to study inclusion (inclusion/exclusion criteria). Additional pregnancy testing should be considered taking into account, amongst others, the duration of the trial.

##### **Evaluate patient eligibility**

## **6.2.2 Baseline measurements**

### **Visit 2: Baseline measurements.**

- May be performed same day as screening. May use screening measurements as baseline values if screening was undertaken within 21 days prior to treatment. May involve several visits (for instance, cardiac MRI on a separate day).

#### **Clinical status**

- Refer to Section 6.2.1.

#### **Concomitant medication**

- Refer to Section 6.2.1.

#### **Laboratory analysis**

- Blood samples: CRP, Hb, WBC (incl. differential counting), PLT, Na, K, Ca, creatinine, ASAT, ALAT, LD, ALP,  $\gamma$ GT, INR, albumin, bilirubin, cholesterol fractions and triglycerides.
- Biobanking of blood, plasma and serum
- OGTT
- Urine samples: Stix for glucose, blood, protein. Albumine-creatinine ratio

#### **Women of childbearing potential**

Refer to Section 6.2.1.

**Re-evaluate patient eligibility** if Screening was undertaken >21 days prior to treatment:

#### **Assess baseline values of parameters used as end-points**

- Office BP x 3
- OGTT
- Cardiac MRI
- 24h BP (started before or at this visit)

#### **Enrolment**

- Randomization (from here the trial subject is considered enrolled and part of the ITT population)
- Dispensation of IMP (treatment is not to be started until baseline measurements are completed, i.e. after 24h BP recording is finished)

## **6.2.3 During Treatment**

### **Visit 3: Day 14 $\pm$ 7 Safety visit**

- Blood samples: CRP, Hb, WBC (incl. differential counting), PLT, Na, K, Ca, creatinine, ASAT, ALAT, LD, ALP,  $\gamma$ GT, INR, albumin, bilirubin.
- Record adverse events
- Record of concomitant medications (incl. "over-the-counter" drugs)

### 6.2.4 End of Study Visit

#### Visit 4: Day 275 (-30 to + 60) End of Study Visit

- The following recordings/procedures may be performed on separate days if necessary. The IMP is preferentially continued until completion of outcome assessments.
- Physical examination eg. (cor/pulm/abdomen and peripheral lymph node status), vital signs (weight, blood pressure, temperature and pulse)
- Record of concomitant medications (incl. "over-the-counter" drugs) and lost doses of IMP from drug diary
- Blood samples: CRP, Hb, WBC (incl. differential counting), PLT, Na, K, Ca, creatinine, ASAT, ALAT, LD, ALP, γGT, INR, albumin, bilirubin, cholesterol fractions and triglycerides.
- Biobanking of blood, plasma and serum
- Urine samples: Stix for glucose, blood and protein. Albumine-creatinine ratio
  
- Office BP x 3
- OGTT
- Cardiac MRI
- 24h BP (started before or at this visit)
  
- Record adverse events
- Return of used and unused pill boxes and medical diary

### 6.2.5 Withdrawal Visit

#### Visit X: Withdrawal Visit

- All subjects that participate in the trial have the right to withdraw from the study at any time.
- The reason for withdrawal / discontinuation must be recorded in the CRF if the trial subject agrees to.
- If the trial subject agrees to, a clinical visit may be arranged, preferentially including the following
  - Record adverse events
  - Record of concomitant medications (incl. "over-the-counter" drugs) and lost doses of IMP from drug diary
  - Physical examination eg. (cor/pulm/abdomen and peripheral lymph node status), vital signs (weight, blood pressure, temperature and pulse)
  - Blood samples: CRP, Hb, WBC (incl. differential counting), PLT, Na, K, Ca, creatinine, ASAT, ALAT, LD, ALP, γGT, INR, albumin, bilirubin, cholesterol fractions, triglycerides, uric acid
  - Urine samples: Stix for glucose, blood and protein. Albumin-creatinine ratio
- Any significant adverse events should be followed until the outcome either is recovered or resolved, recovering/resolving, not recovered/not resolved, recovered/resolved with sequelae, fatal or unknown.
- Withdrawal due to non-attendance will be followed up by the Investigator. For all patients, including patients who withdraw from the study, all documentation should be as complete as possible.

## 6.3 Criteria for Patient Discontinuation

Patients may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a patient for this study are:

- Voluntary discontinuation by the patient who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment.
- Safety reason as judged by the Principal Investigator
- Major protocol deviation
- Incorrect enrolment ie, the patient does not meet the required inclusion/exclusion criteria for the study
- Patient lost to follow-up
- A female patient becoming pregnant
- Patient's non-adherence to study treatment and/or procedures

## 6.4 Procedures for Discontinuation

### 6.4.1 Patient Discontinuation

Trial subjects who withdraw or are withdrawn from the study, will stop further treatment. Please refer to Section 6.2.4 "withdrawal visit" for details. In summary: If the trial subjects agree to

- The reason for discontinuation shall be recorded
- A withdrawal visit should be arranged

The investigator is obliged to follow up any significant adverse events until the outcome either is recovered or resolved, recovering/resolving, not recovered/not resolved, recovered/resolved with sequelae, fatal or unknown.

Patient's who withdraw or are withdrawn from the study before randomization, will be replaced.

### 6.4.2 Trial Discontinuation

The whole trial may be discontinued at the discretion of the PI / sponsor in the event of any of the following:

- Occurrence of AEs unknown to date in respect of their nature, severity and duration
- Medical or ethical reasons affecting the continued performance of the trial
- Difficulties in the recruitment of patients

The PI / sponsor will inform all investigators, the relevant Competent Authorities and Ethics Committees of the termination of the trial along with the reasons for such action. If the study is terminated early on grounds of safety, the Competent Authorities and Ethics Committees will be informed within 15 days.

## 6.5 Laboratory Tests

Blood samples (CRP, Hb, WBC [incl.differential counting], PLT, Na, K, Ca, creatinine, ASAT, ALAT, LD, ALP, γGT, INR, albumin, bilirubin, cholesterol fractions, triglycerides, uric acid) and urine samples (stix for glucose, blood and protein, and albumin-creatinine ratio) will be collected. These are all routine samples that will be collected, handled and analysed at the Department of Medical Biochemistry in accordance with hospital/laboratory standard procedures. Biobanking of blood will be performed for measurement of uric acid and for post hoc analyses. Samples for the biobank include 4 ml EDTA full blood, 4 ml of serum (w/o gel) and 4 ml of EDTA plasma. After 30-120 minutes the two latter blood samples are centrifuged at 3250 rpm for 10 minutes and the serum and plasma separated and each aliquoted in 4 cryo vials. All samples are frozen at -80 degrees Celcius.

## 7 ASSESSMENTS

### 7.1 Assessment of Efficacy Response

Assessments for the following efficacy parameters will be determined both at baseline and at end of treatment (i.e. 9 months):

#### **Left ventricular mass**

Examinations will be performed on a Siemens 1.5 T MR unit at Oslo University Hospital, Aker, using a phased array coil.

#### **Ambulatory blood pressure**

24-hour ambulatory blood pressure will be measured with the Oscar2 apparatus (SunTech Medical, Inc. Morrisville, NC U.S.A). Mean overall systolic and diastolic blood pressure will be used.

#### **Office blood pressure**

Office blood pressure will be measured seated after ten minutes rest by Dyna Map (Tuff.-Cuff, CAS Medical system Inc.) and the mean of the two last out of three measurements will be used.

#### **Insulin sensitivity and insulin release**

The metabolic clearance rate of glucose, insulin sensitivity index, 1<sup>st</sup> and 2<sup>nd</sup> phase of insulin release will be estimated according to Stumvoll et al. using an oral glucose tolerance test (1). Measurements of plasma glucose (G) and insulin (I) before (G+I), 30 (G+I), 90 (G) and 120 (G+I) minutes following an oral administration of 75 g glucose dissolved in 3 dl water.

#### **Other**

Serum uric acid (from biobanked serum), urinary albumin excretion rate and estimated glomerular filtration rate will be measured in accordance with laboratory standard procedures. The glomerular filtration rate is estimated from creatinine values, using the CKD-EPI formula (2).

### 7.2 Safety and Tolerability Assessments

Safety will be monitored by the assessments described below as well as the collection of AEs at every visit. Significant findings that are present prior to the signing of informed consent must be included in the relevant medical history/ current medical condition page of the CRF.

For details on AE collection and reporting, refer to Section 8, and for the expected AEs to Section 8.2..

For the assessment schedule refer to Flow chart in Section 6.1.

A safety visit at week 2 will include assessment of blood samples to detect side effects from allopurinol: CRP, Hb, WBC (incl. differential counting), PLT, Na, K, Ca, creatinine, ASAT, ALAT, LD, ALP, γGT, INR, albumin, bilirubin.

Trial participants will be offered clinical visit if AE is suspected at other time points during the study.

## 8 SAFETY MONITORING AND REPORTING

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE). Each patient will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

The methods for collection of safety data are described below.



## 8.1 Definitions

### 8.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

The term AE is used to include both serious and non-serious AEs.

Expected AE from treatment with allopurinol are those side effects listed in the SmPC will be recorded in the CRF, listed in the annual and final report according to guidelines.

If an abnormal laboratory value/vital sign are associated with clinical signs and symptoms, the sign/symptom should be reported as an AE and the associated laboratory result/vital sign should be considered additional information that must be collected on the relevant CRF.

### 8.1.2 Serious Adverse Event (SAE)

Any untoward medical occurrence that at any dose:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

Medical and scientific judgment is to be exercised in deciding on the seriousness of a case. Important medical events may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the listed outcomes in the definitions above. In such situations, or in doubtful cases, the case should be considered as serious. Hospitalization for administrative reason (for observation or social reasons) is allowed at the investigator's discretion and will not qualify as serious unless there is an associated adverse event warranting hospitalization. Refer also to Section 8.5 for the distinction between severe and serious AE.

### 8.1.3 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Adverse Reaction: all untoward and unintended responses to an investigational medicinal product related to any dose administered;

Unexpected Adverse Reaction: an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

Suspected Unexpected Serious Adverse Reaction: SAE (see section 8.1.2) that is unexpected as defined in section 8.2 and possibly related to the investigational medicinal product(s)..

## 8.2 Expected Adverse Events

Allopurinol is generally well tolerated, but approximately 2% develop mild rash, and up to 5% stop treatment due to any adverse event (53). Expected AE from treatment with allopurinol are those side effects listed in the SmPC, including (but not restricted to) pruritus, skin eruptions, leucopenia with or without infection, abdominal pain, nausea, diarrhoea, pathologic liver function tests.

Allopurinol hypersensitivity syndrome (AHS) is a rare (~1/1000) but potentially fatal complication which may present by rash (Stevens-Johnson syndrome, toxic epidermal necrolysis), eosinophilia, leukocytosis, fever, hepatitis and renal failure (54). There is no cure; early diagnosis and withdrawal of treatment are important. AHS is per definition an expected AE, although the risk of such an occurrence would be very small in our study with 30 trial subjects exposed to allopurinol (about 3% risk of any occurrence  $[1-0.999^{30}]$ ).

Expected AEs need not be reported acutely to REK or SLV, but should be recorded in the CRF.

## 8.3 Time Period for Reporting AE and SAE

For each patient the standard time period for collecting and recording AE and SAEs will begin at start of study treatment and will continue until the last day of study treatment for each patient.

During the course of the study all AEs and SAEs will be proactively followed up for each patient; events should be followed up to resolution, unless the event is considered by the investigator to be unlikely to resolve due to the underlying disease. Every effort should be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion.

## 8.4 Recording of Adverse Events

If the patient has experienced adverse event(s), the investigator will record the following information in the CRF:

- The nature of the event(s) will be described by the investigator in precise standard medical terminology (i.e. not necessarily the exact words used by the patient).
- The duration of the event will be described in terms of event onset date and event ended data.
- The Causal relationship of the event to the study medication will be assessed as one of the following:

### **Unrelated:**

There is not a temporal relationship to investigational product administration (too early, or late, or investigational product not taken), or there is a reasonable causal relationship between non-investigational product, concurrent disease, or circumstance and the AE.

### **Unlikely:**

There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between the investigational product and the AE.

### **Possible:**

There is reasonable causal relationship between the investigational product and the AE. Dechallenge information is lacking or unclear.

### **Probable:**

There is a reasonable causal relationship between the investigational product and the AE. The event responds to dechallenge. Rechallenge is not required.

**Definite:**

There is a reasonable causal relationship between the investigational product and the AE.

- Action taken
- The outcome of the adverse event – whether the event is resolved or still ongoing.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 8.1. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but is not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

## 8.5 Reporting Procedure

### 8.5.1 AEs and SAEs

All adverse events and serious adverse events that should be reported as defined in section 8.1.1 will be recorded in the patient's CRF.

SAEs must be reported by the investigator to the sponsor, **Dag Olav Dahle**, Tel: +47 23 07 35 44, within 24 hours after observation of the SAE by study personell. Every SAE must be documented by the investigator on the SAE pages (to be found in the investigator site file). The Serious Adverse Event Report Form must be completed, signed and sent in according to guidelines to Dag Olav Dahle, Nyrefysiologisk laboratorium, Oslo University Hospital Rikshospitalet, Department of Transplant Medicine, Pb 4950 Nydalen, 0424 Oslo. The initial report shall promptly be followed by detailed, written reports if necessary. The initial and follow-up reports shall identify the trial subjects by unique code numbers assigned to the latter.

The sponsor keeps detailed records of all SAEs reported by the investigators and performs an evaluation with respect to seriousness, causality and expectedness.

### 8.5.2 SUSARs

SUSARs will be reported to the Competent Authority and Ethics Committee according to national regulation. The following timelines should be followed:

The sponsor will ensure that all relevant information about suspected serious unexpected adverse reactions that are fatal or life-threatening is recorded and reported as soon as possible to the Competent Authority and Ethics Committee in any case no later than seven (7) days after knowledge by the sponsor of such a case, and that relevant follow-up information is subsequently communicated within an additional eight (8) days.

All other suspected serious unexpected adverse reactions will be reported to the Competent Authority concerned and to the Ethics Committee concerned as soon as possible but within a maximum of fifteen (15) days of first knowledge by the sponsor.

SUSARs will be reported using the CIOMS form since Oslo University Hospital is not connected to EudraVigilance.

### 8.5.3 Annual Safety Report

Once a year throughout the clinical trial, the sponsor will provide the Competent Authority with an annual safety report. The format will comply with national requirements.

### 8.5.4 Clinical Study Report

The adverse events and serious adverse events occurring during the study will be discussed in the safety evaluation part of the Clinical Study Report.

## **8.6 Procedures in Case of Emergency**

The investigator is responsible for assuring that there are procedures and expertise available to cope with emergencies during the study.

In case of critical clinical circumstances, upon the discretion of the principal investigator, the treatment code of a specific patient will be allowed to be unblinded.

## **9 DATA MANAGEMENT AND MONITORING**

### **9.1 Case Report Forms (CRFs)**

The designated investigator staff will enter the data required by the protocol into the Case report forms (CRF). The Principal Investigator is responsible for assuring that data entered into the CRF is complete, accurate, and that entry is performed in a timely manner. The signature of the investigator will attest the accuracy of the data on each CRF. If any assessments are omitted, the reason for such omissions will be noted on the CRFs. Corrections, with the reason for the corrections will also be recorded.

### **9.2 Source Data**

The medical records for each patient should contain information which is important for the patient's safety and continued care, and to fulfill the requirement that critical study data should be verifiable.

To achieve this, the medical records of each patient should clearly describe at least:

- That the patient is participating in the study, e.g. by including the enrollment number and the study code or other study identification;
- Date when Informed Consent was obtained from the patient and statement that patient received a copy of the signed and dated Informed Consent;
- Results of all assessments confirming a patient's eligibility for the study;
- Diseases (past and current; both the disease studied and others, as relevant);
- Treatments withdrawn/withheld due to participation in the study;
- Results of assessments performed during the study;
- Treatments given, and changes in treatments noted at the study visits;
- Visits to the clinic / telephone contacts during the study, including those for study purposes only;
- Non-Serious Adverse Events and Serious Adverse Events (if any) including causality assessments;
- Date of, and reason for, discontinuation from study treatment;
- Date of, and reason for, withdrawal from study;
- Date of death and cause of death, if available;
- Additional information according to local regulations and practice.

For the outcome and adherence data, the source data will be recorded directly into the Case Report Form.

### 9.3 Study Monitoring

The investigator will be visited on a regular basis by the Clinical Study Monitor, who will check the following:

- Informed consent process (100%)
- Reporting of adverse events (100%)
- Reporting safety data
- Adherence to protocol
- Maintenance of required regulatory documents
- Study Supply accountability
- Data completion on the CRFs including source data verification (SDV).

The monitor will review the relevant CRFs for accuracy and completeness and will ask the site staff to adjust any discrepancies as required. The monitor will check the first 3 study subjects completely and thereafter a random check of 10% of the data on bullet points 3 and forward (see above). If major issues are observed another 3 patients are to be completely checked and the algorithm continued in the same manner.

When the responsible study monitor has checked and verified the CRFs, the data will be entered into a computer database at the Oslo University Hospital Rikshospitalet's scientific server for further handling and statistical evaluation.

Sponsor's representatives (e.g. monitors, auditors) and/or competent authorities will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study may be required.

### 9.4 Confidentiality

The investigator shall arrange for the secure retention of the patient identification and the code list. Patient files shall be kept for the maximum period of time permitted by each hospital. The study documentation (CRFs, Site File etc) shall be retained and stored during the study and for 15 years after study closure. All information concerning the study will be stored in a safe place inaccessible to unauthorized personnel.

### 9.5 Database management

Trial subjects will receive a unique identification number, which will be linked to the CRF. The data will then be blinded (NO: "avidentifisert") correspondingly in all data analyses. Reference to where details of data provided can be found (what is the source document) will be defined in CRF. Study documentation will be stored for at least 15 years after study completion, in accordance with current guidelines.

## 10 STATISTICAL METHODS AND DATA ANALYSIS

### 10.1 Determination of Sample Size

Calculations assume a t-test of differences in change scores between treatment arms, two-sided alpha 0.05 and power 0.8. For changes in left ventricular mass, assuming an SD of 5 g (reference (25) and a 3.8 g expected difference in change (reference (23-25), 27 patients would be required in each study arm. To allow for 30% drop-out (patient discontinuation), 40 persons will be targeted in each group. Assuming a group size of 27, power is 0.75 to detect a change of 5 mmHg in ambulatory blood pressure (assuming an SD of 7; reference (10, 31, 32). We do not have available data on repeated measurements of insulin sensitivity estimated according to Stumvoll et al (1), but using data

from repeated measurements with the gold-standard hyperinsulinemic-euglycemic clamp, assuming an SD of 2 mg/kg per min and a difference in change of 2 mg/kg per min (comparable to the effect of exercise; reference (55), power is 0.96, indicating power is likely also adequate for estimated insulin sensitivity.

## **10.2 Randomization**

### **10.2.1 Allocation- sequence generation**

The allocation of treatment sequence will be performed by the R-package “blockrand” using simple randomization, one block and equal probabilities of each sequence. A person not directly involved in the practical implementation of the study will perform the allocation. Numbered envelopes with the allocated drug package (active and placebo, blinded).

### **10.2.2 Allocation- procedure to randomize a patient**

For each new patient to start on study drug treatment the next randomization envelope will be opened and patient assigned the listed drug package.

### **10.2.3 Blinding and emergency unblinding**

Both patient and investigator/study personell is blinded with regards to study drug (active/placebo) during the study period. Data manager and analyst will also be kept blinded during the analysis period. The code will be opened after the completion of all analyses outlined in the protocol. Study drugs (active/placebo) will be prepared and marked before shipment to the Pharmacy at the investigator site.

Sealed numbered envelopes containing the treatment group for each participant will be stored at OUS Rikshospitalet in an office with restricted access. Medical doctors are allowed entry by ID card. In case of critical clinical circumstances, upon the discretion of the principal investigator, the treatment code of a specific patient will be allowed to be unblinded. Outside office hours, the principal investigator can be contacted through the switchboard at OUS Rikshospitalet or OUS Ullevål, and will guide the medical doctor on call at OUS Rikshospitalet to the location of the sealed envelopes.

## **10.3 Population for Analysis**

The following populations will be considered for the analyses:

- Intention to treat (ITT) population: All randomized participants, regardless of protocol adherence.
- Per-protocol population (PP): Includes all subjects who have adhered to 9 months treatment.
- Safety population: Includes all subjects who have received at least one dose of study medication. Subjects who withdraw from the study will be included in the safety analysis. A list of withdrawn subjects, preferably with the reasons for withdrawal, will be made.

## **10.4 Planned analyses**

The main statistical analysis is planned when all patients have completed the study. Deviation from the original statistical plan will be described and justified in the Clinical Study Report.

## **10.5 Statistical Analysis**

1. The primary analysis variable will be left ventricular mass (LVM) assessed with MRI. Change from baseline to 9 months will be analyzed in a linear regression model using the LVM at 9 months as dependent variable and as

independent variables baseline LVM and randomization group (56). The study hypothesis is that the change in LVM (follow-up minus baseline value) will be less positive (alternatively more negative) in the allopurinol group than the placebo group after 9 months of treatment. A two-sided P-value of 0.05 will be used. The primary analysis will be in the PP population. Sensitivity analyses will include adjustment for other baseline variables, including age, gender, kidney function and systolic and diastolic blood pressure, and change in systolic and diastolic blood pressure.

2. The secondary outcomes will be analyzed in a similar way as delineated for the primary outcome. The analysis variables will be systolic and diastolic ambulatory BP, systolic and diastolic office BP, estimated metabolic clearance rate of glucose, estimated insulin sensitivity index, estimated first-phase insulin release, estimated second-phase insulin release, serum uric acid levels, urinary albumin excretion rate, estimated glomerular filtration rate. Decrease in number and doses of antihypertensive medications will be compared using Fisher's exact test.

Demographical data will be summarized using means, medians, minimums, maximums and standard deviations for normally distributed variables and frequency counts and percent for categorical variables.

### **10.5.1 Analysis of Safety**

Adverse events (AEs) in the safety population will be tabulated and compared.

## **11 STUDY MANAGEMENT**

### **11.1 Investigator Delegation Procedure**

The principal investigator is responsible for making and updating a "delegation of tasks" listing all the involved co-workers and their role in the project. He will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

### **11.2 Protocol Adherence**

Investigators ascertain they will apply due diligence to avoid protocol deviations.

All significant protocol deviations will be recorded and reported in the Clinical Study Report (CSR).

### **11.3 Study Amendments**

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol (Amended Protocol) must be notified to and approved by the Competent Authority and the Ethics Committee according to EU and national regulations.

### **11.4 Audit and Inspections**

Authorized representatives of a Competent Authority and Ethics Committee may visit the centre to perform inspections, including source data verification. Likewise the representatives from sponsor may visit the center to perform an audit. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (ICH GCP), and any applicable regulatory requirements. The principal investigator will ensure that the inspectors and auditors will be provided with access to source data/documents.

## **12 ETHICAL AND REGULATORY REQUIREMENTS**

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice and applicable regulatory requirements. Registration of patient data will be carried out in accordance with national personal data laws.

### **12.1 Ethics Committee Approval**

The study protocol, including the patient information and informed consent form to be used, must be approved by the regional ethics committee before enrolment of any patients into the study.

The investigator is responsible for informing the ethics committee of any serious and unexpected adverse events and/or major amendments to the protocol as per national requirements.

### **12.2 Other Regulatory Approvals**

The protocol will be submitted and approved by the applicable competent authorities before commencement of the study.

The protocol will also be registered in [www.clinicaltrials.gov](http://www.clinicaltrials.gov) before inclusion of the first patient.

### **12.3 Informed Consent Procedure**

The investigator is responsible for giving the patients full and adequate verbal and written information about the nature, purpose, possible risk and benefit of the study. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever she/he wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered in the study. This will be done in accordance with the national and local regulatory requirements. The investigator is responsible for obtaining signed informed consent.

A copy of the patient information and consent will be given to the patients. The signed and dated patient consent forms will be filed in the Investigator Site File binder.

### **12.4 Subject Identification**

The investigator is responsible for keeping a list of all patients (who have received study treatment or undergone any study specific procedure) including patient's date of birth and personal number, full names and last known addresses.

The patients will be identified in the CRFs by patient number, initials.

## **13 TRIAL SPONSORSHIP AND FINANCING**

This study is investigator-initiated and solely financed by the investigator committee. The study is sponsored by Oslo Universital Hospital, Rikshospitalet with research grant from the South-Eastern Norway Regional Health Authority (project 2017120).



## **14 TRIAL INSURANCE**

The Principal investigator has insurance coverage for this study through membership of the Drug Liability Association (see <http://www.laf.no> for more details).

## **15 PUBLICATION POLICY**

Upon study completion and finalization of the study report the results of this study will either be submitted for publication and/or posted in a publicly assessable database of clinical study results.

The results of this study will also be submitted to the Competent Authority and the Ethics Committee according to EU and national regulations.

All personnel who have contributed significantly with the planning and performance of the study (Vancouver convention 1988) may be included in the list of authors.

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## **17 LIST OF APPENDICES**

- A Key studies
- B Drug labeling

## APPENDIX A – KEY STUDIES

Group	N	Dose	FU	SUA	ΔSUA	LVM	ΔLVM	SBP	ΔSBP	DBP	ΔDBP	HOMA	ΔHOMA	Ref
<b>Observational studies of kidney donors – effect on LVM</b>														
Donor	50	N/A	12 mth	276	57	114	6.8±9.5*	124	-1.2±10.2	75	2.7±7.7			(10)
Control	45	N/A	12 mth	298	2	117	-2.9±7.6	124	-2.8±7.8	76	-0.04±8.1			
Donor	23	N/A	12 mth			112	3.1±1.4†	129	2±14	76	0±11			(11)
<b>RCTs of allopurinol – effect on LVM</b>														
CKD	27	300	9 mth	440	-180	61.6 <sup>§</sup>	-1.4±4.7 <sup>§</sup>	139	-6.9±14.4	70	-3.3±7.8			(23)
	26	placebo	9 mth	420		62.1 <sup>§</sup>	1.3±4.5 <sup>§</sup>	145	-5.1±15.1	75	-2.5±9.1			
IHD	27	600	9 mth	590	-270	145.7	-5.2±5.8	135	-5±13	78	-4.2±8			(25)
	28	placebo	9 mth	560		136.4	-1.3±4.5	134	-3±15	76	-1.4±8			
DM2	26	600	9 mth	550	-270	125.6	-2.7±5.9	141	0.5±15	77	-7.2±8.4			(24)
	29	placebo	9 mth	540	-19	119.9	1.21±5.1	137	-6.5±29	79	-4.5±13			
<b>RCTs of allopurinol – effect on BP and insulin resistance</b>														
HTN 1	30	400	4 wk	416	-166			137	-6.9 (-4.5 to -9.3)*	83	-5.1 (-2.5 to -7.8)*			(31)
	30‡	placebo	4 wk	369	12			139	-2.0(0.3 to -4.3)	82	-2.4 (0.2 to -4.1)			
preHTN	20	400	8 wk	404	-167			128	-10.1 (-8. to -13.1)*	76	-8.0 (-5.8 to -10.1)*			(32)
	20	placebo	8 wk	393	-18			126	1.7 (0.9 to 3.8)	74	1.6 (0.2 to 3.5)			
DM2	82	~234**	3 yr	433	-104			121	2±3*	74	1±3*	3.4	0.10±0.15*	(41)
	70	control	3 yr	432	23			121	6±2	74	4±2	3.4	0.21±0.18	

Observational studies of left ventricular mass in kidney donors and randomized controlled trials of allopurinol treatment. For details refer to chapter 1.1 and 1.3.

\*p<0.05 in between-group comparison. †p<0.05. <sup>§</sup>indexed for body surface area. ‡cross-over. \*\*mean value.

Dose, dose of allopurinol (in mg). FU, follow-up. SUA, serum uric acid (in μmol/L). LVM, left ventricular mass (in grams). SBP, systolic blood pressure (in mmHg). Δ, change. DBP, diastolic blood pressure. HOMA, homeostasis assessment for insulin resistance. N/A not applicable. CKD, chronic kidney disease. IHD, ischemic heart disease. DM2, diabetes type 2. BP, blood pressure. HTN 1, grade 1 hypertension. preHTN, pre-hypertension.

## APPENDIX B – DRUG LABELING

The IMP will have a label permanently affixed to the outside and will be labeled according to ICH GCP and national regulations, stating that the material is for clinical trial / investigational use only and should be kept out of reach of children. Documentation for the labeling follows below, except for the example labels ("Etikettprøver"), which will be provided later.

Etikettforslag nr 1 Allopurinol 300 mg/Placebo  
«AL-DON»  
Studiekode: 2017-000666-30

### Etikett-del 1): Felles etikett-del (lik for alle enhetene)

TIL KLINISK UTPRØVING  
Studiekode: 2017-000666-30  
Utpøver: Lege Dag Olay Dahle  
Nyreseksjonen  
OUS, Rikshospitalet  
Sognsvannsvei. 20, 0372 Oslo  
Tlf: +47 23070000/22113080

### Etikett-del 2) Pasientspesifikk del.

\*Stolpe: 112 kapsler Allopurinol 300 mg/Placebo  
(vertikal) Pasientnr.: Boks 1 av 3  
BRUKSANVISNING  
1 kapsel daglig  
Tas i forbindelse med måltid  
Oppbevares tørt og lysbeskyttet  
i utleveringsemballasjen og ved  
temperatur mellom +15 og 25°C  
Oppbevares utilgjengelig for barn

\*Stolpen: (På pasientspesifikk del – vertikalt i venstre side)  
Kragers Tablettproduksjon as  
P.nr.: (for begge preparatene)  
Utløpsdato:

I tillegg settes en streif-etikett «Svelges hele» på hver enhet


### MERK:

Etiketten blir en felles etikett 40 x 120 mm med den Pasientspesifikke delen (Etikett 2) i venstre side og høyre del av etiketten er Fellesetiketten (nr. 1). Det er et klart skille mellom de to etikett-delene.

\*Det utleveres 3 bokser/pasient og boksene merkes «Boks 1 av 3, 2 av 3 og 3 av 3»

Etikettprøver:

Kragers, 10. februar 2017

  
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